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STUDIES ON CHANGES IN THE DEGREE OF OXIDATION OF ARSENIC IN ARSENICAL DIPPING BATHS.

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INTRODUCTORY.

In the approximately four years during which arsenical dipping baths have been extensively employed for eradicating the Texas-fever tick in this country, and in the even longer period during which they have been put to similar use abroad, it has been discovered, along with other interesting and important facts, that the arsenic in such baths is prone to change its degree of oxidation. Arsenic is one of the considerable number of chemical elements which may be said to lead double lives; that is, they form two distinctly different series of compounds in which the element acts in many respects as if it were indeed two wholly different elements. Thus the element arsenic forms two oxids, first, arsenious oxid, in which two atoms of arsenic are combined with three atoms of oxygen, and, second, arsenic oxid, in which two atoms of arsenic are combined with five atoms of oxygen. Both these oxids are essentially acid in nature; that is, they readily combine with alkaline substances (bases) to form salts. For example, when arsenious oxid is treated with sodium carbonate or sodium-hydroxid there is produced sodium arsenite, while arsenic oxid, similarly treated, yields sodium arsenate. The common white arsenic of commerce is merely arsenious oxid. So when in the ordinary preparation of dipping baths, white arsenic is heated with a solution of caustic soda or of carbonate of soda, the resulting liquid is essentially simply a solution of sodium arsenite.

Soon after arsenical dipping baths had come into extensive practical use it was discovered that the sodium arsenite frequently seemed to disappear slowly with continued use of the bath. Thence it was only one step to discover that the element arsenic was in fact

NOTE.—This bulletin will be of interest to cattle raisers who employ arsenical dipping baths in eradicating the Texas-fever tick.

still present in its original quantity but that it had partly changed its form and now existed to that extent as sodium arsenate,¹ a compound, as previously indicated, quite different in many of its properties from sodium arsenite. Fuller,² who independently discovered the phenomenon in the laboratories of the Bureau of Animal Industry, went further and showed that the change is essentially brought about through the growth of microorganisms in the dipping baths, other factors being relatively of little importance.

After the appearance of Fuller's paper pressure of other work prevented the obtaining of further knowledge along this line except what could be gained from the routine examination of samples from the field and an occasional desultory experiment. What was thus learned, however, indicated that the matter was probably not yet thoroughly understood and that at any rate practical studies should certainly be undertaken on the progress of the phenomenon of oxidation as it occurs in dipping vats in the field under varied conditions of actual use. The relative efficacy of solutions of arsenite and arsenate against ticks and their relative toxicity toward animals dipped therein are subjects entirely beyond the scope of this paper, but it is universally believed that sodium arsenite is a much more powerful tickicide than sodium arsenate, though at the same time more poisonous to cattle. It was clearly a matter of importance to ascertain how rapidly a change from one form to the other might go on in baths in actual use in the field, since such a change is necessarily closely related to the regularity of action of a bath upon both ticks and cattle. These field studies have been continued during the past two years.

In the meantime further published work has appeared abroad. Laws³ in explanation of certain observations suggested that, in addition to the oxidizing organisms already noted which change arsenite to arsenate, there may also exist in some baths reducing organisms which change arsenate back to arsenite. He also suggested the use of some bactericide to prevent oxidation by preventing the growth of the bacteria which cause it. Brännich and Smith,⁴ Williams,⁵ and Holborow⁶ have made some studies of the obvious questions presented. But the published work so far seems in general hardly comprehensive enough to insure the drawing of sound practical conclusions, while in any event it is confined to certain foreign countries where dipping practice as respects strength of dip and

¹ Brännich (Australian Association for the Advancement of Science, 1909, through *Journal of Agricultural Science*, 1911, vol. 4, p. 177) appears to have been first to make this discovery.

² Bureau of Animal Industry Circular 182, Nov. 9, 1911.

³ *Agricultural Journal of Union of South Africa*, 1913, vol. 5, p. 915.

⁴ *Queensland Agricultural Journal*, 1914, vol. 2, part I, p. 81.

⁵ *Agricultural Journal of Union of South Africa*, 1913, vol. 5, p. 68; 1914, vol. 8, p. 12.

⁶ *Rhodesia Agricultural Journal*, 1914, vol. 11, p. 579.

intervals between dipping is so different from ours as to be scarcely at all comparable.

FIELD EXPERIMENTS OF 1913.

The experiments of 1913 involved the taking at fortnightly intervals of samples from a number of vats, under as varied conditions of use as possible. That is, the vats were in different sections of the country; some were large vats at stock-yards centers, through which hundreds of cattle were passed daily; others were small vats in thinly settled localities, where only a few cattle were dipped at infrequent intervals. The samples were tested at the vat by the bureau field method,¹ were tightly sealed with wax or paraffin to prevent, by exclusion of air, oxidation en route, and were analyzed at the laboratory. Unfortunately the methods of analysis since published¹ had not then been completely worked out, for kaolin was used as a clarifying agent instead of blood-charcoal. The results, therefore, are not of the highest accuracy, but are sufficiently correct for the purpose of making the comparative studies desired. Also, the possibility of reduction by bacteria en route was not then suspected and hence no provision was made for its prevention. For the figure for actual arsenious oxid it was therefore necessary to rely in the majority of cases upon the reported result of the field test, which again is not of high accuracy.

For the purpose of making comparisons between different vats it is necessary to compare the extent to which they were used, and since vats are of very different sizes and cattle are dipped at varying intervals, it is necessary to reduce the corresponding figures to a common denominator. The "figure of use" (abbreviated "F. U."), therefore, is a figure which stands for the number of cattle dipped per 100 gallons of bath (capacity of vat) per fortnight.

The results afford the desired information most clearly and with sufficient accuracy when plotted. In the diagrams (figs. 1 and 2) the weeks during which each vat was in use are laid off horizontally, and the percentages of arsenious oxid, found by analysis, are laid off vertically. The large shaded circles where the lines begin to diverge indicate the points at which the vats were filled with entirely fresh bath; the small circles on the lines indicate the results actually obtained by analysis. The circles lying on the full lines indicate percentages of actual arsenious oxid; that is, arsenic in the form in which it was originally introduced. The circles lying on the broken lines indicate percentages of "total arsenious oxid," which includes all the arsenious oxid present, whether in its original condition or after undergoing oxidation to arsenic oxid.

¹ See Department of Agriculture Bulletin 76.

The vats were subject to all the vicissitudes of ordinary use. They sometimes lost water through evaporation and sometimes gained it through entrance of rain water. The inspectors in charge added water, chemicals, or both, whenever in their judgment the strength or volumes of the baths needed correction. Naturally, therefore,

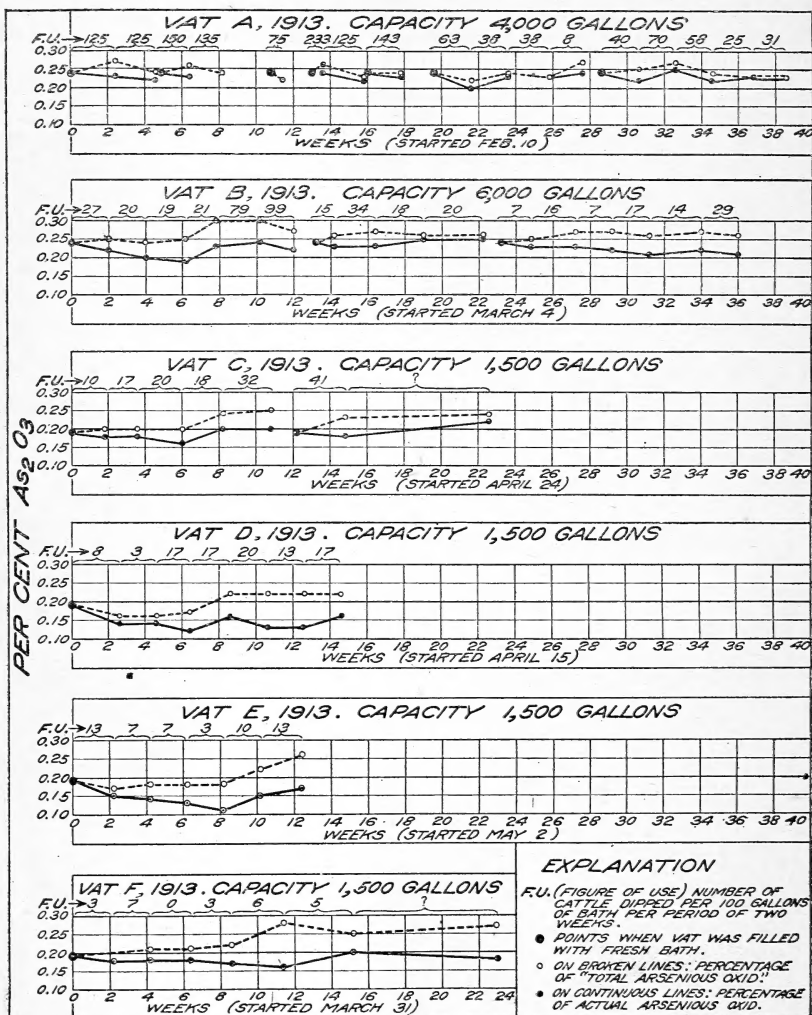


FIG. 1.—Diagram (vats A to F) showing changes in oxidation of arsenic in dipping baths.

the straight course of either the full or broken lines connecting each two consecutive circles does not mean that the composition of the dip changed gradually from point to point. The lines are introduced merely to guide the eye from one circle to the next.

The diagrams show, first, that all vats tend to become oxidized immediately or shortly after they have been put into use. This tendency may persist indefinitely, and the lines may thus spread farther and farther apart. Or, secondly, in some cases after a certain period of use, the first tendency to become oxidized may become diminished or overbalanced by a tendency in the opposite direction, so that the lines thereafter remain parallel or even converge. In a general way the

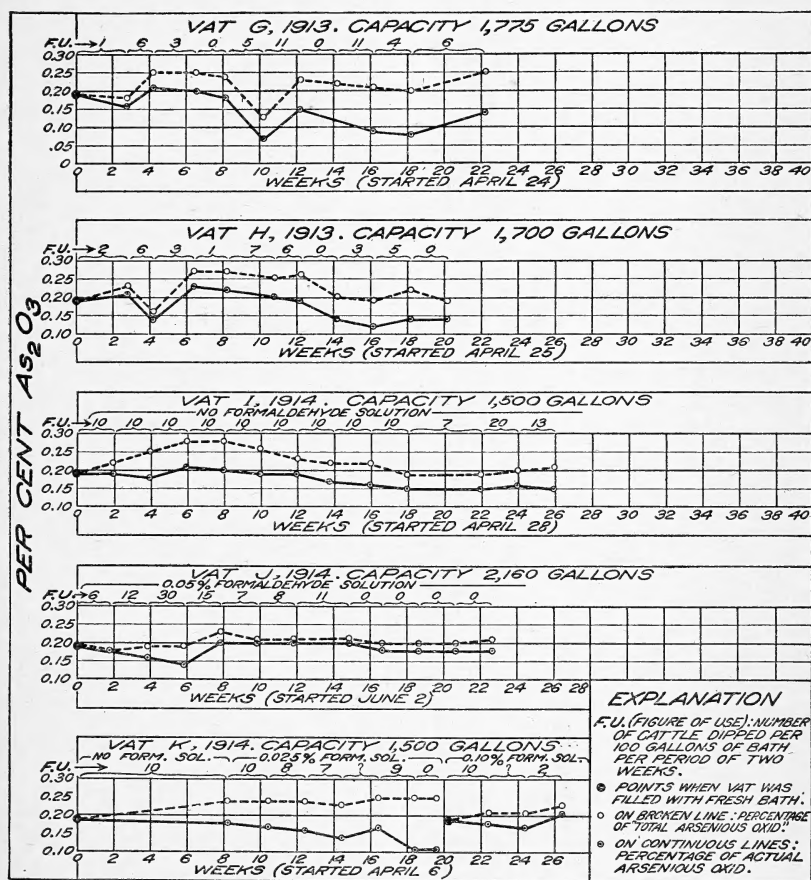


FIG. 2.—Diagram (vats G to K) showing changes in oxidation of arsenic in dipping baths.

size of the figure of use (the diagrams for 1913 are purposely arranged in the descending order of the average figures of use of the vats) evidently measures the rapidity with which the first stage, that of oxidation, progresses, and also the rapidity with which the second stage, that of reduction, first appears and the extent to which it thereafter inhibits or overcomes the original tendency to oxidation. Unfortunately, through failure of the field agents of the bureau to

send samples, many of this set of experiments were discontinued at just about the time when the second stage probably had been reached, but the phenomenon will be better illustrated in the series of experiments in 1914.

In explaining the occurrence of the second stage in the history of the vats which show it, it is not enough to say that the considerable degree of usage to which the vats were subjected and the consequent copious renewal with fresh bath allowed no time for the arsenic to become largely oxidized before it had been carried out by the cattle, for in several cases it is very plain that the lines tend to converge under a figure of use that is no greater but even decidedly less than the figure of use under which they had previously diverged, a fact explicable only by the existence in the former case of some agent which positively prevents oxidation. During the examination of samples from vat A, it happened several times that the field test made at the vat by the field agent of the bureau would be reported as showing a figure for actual arsenious oxid decidedly lower than the figure obtained through laboratory analysis, while in some such cases laboratory analysis showed no difference at all between the results for actual and total arsenious oxid; that is, the bath appeared to be entirely unoxidized. While the laboratory was puzzling over this annoying phenomenon and contemplating active steps to investigate the cause, the previously noted article by Laws came to its attention. If Laws's conjecture of the action of reducing bacteria is correct, then it was possible that in the case of these doubtful samples the analyses of the field agent and the laboratory were in fact both correct; that is, the composition of the sample had changed en route. Therefore, laboratory experiments were undertaken to determine the truth of Laws's conjecture, which was not supported by any direct experimental evidence. At the same time it seemed desirable also to run some experiments on oxidation.

LABORATORY EXPERIMENTS.

The plan of investigation was simple. Strong stock solutions of sodium arsenite and sodium arsenate were prepared by boiling the corresponding oxids of arsenic with sodium carbonate in amounts proportionate to the regular bath formula. Nutrient broth was prepared according to the formula, 5 grams solid meat extract, 10 grams Witte's peptone, and $2\frac{1}{2}$ grams sodium chlorid per liter, but sterilization appeared unnecessary for the work in view. For the tests the stock arsenic solutions were diluted, mixed with broth (about 30 per cent of the final volume), antiseptic added if desired, and the mixture inoculated with a portion of used dipping-bath from the field. No tar was added. The freshly made mixtures

were analyzed for arsenic in its two forms and were then left at room temperature for varying lengths of time, after which they were again analyzed. Some tests were carried on in open vessels (beakers) and in these the liquid was kept up to its original level or weight by daily additions of water to replace loss by evaporation.

The following detailed experiments are selected as typical from the considerable number actually performed.

Experiment 1.—To determine possible influence of high initial percentages of actual arsenious oxid upon oxidation. Total initial volumes, 250 c. c., containing 10 c. c. used bath as inoculum. Carried out in open 800 c. c. beakers, replacing loss by evaporation.

TABLE 1.—*Influence of high initial percentages of actual arsenious oxid upon oxidation.*

No. of test.	Per cent actual As_2O_3 by analysis.			
	Original.	10 days.	20 days.	30 days.
a.....	0.200	0.184	0.002
b.....	.240	.234	.068	0.001
c.....	.278	.276	.138	.002
d.....	.316	.312	.146	.002
e.....	.356	.340	.176	.002
f.....	.394	.362	.232	.002

Experiment 2.—To determine possible influence of high initial percentages of total arsenious oxid upon oxidation. Total initial volumes, 250 c. c., containing identical initial amounts of actual arsenious oxid, but varying amounts of arsenic oxid. Inoculated and carried out similarly to experiment 1.

TABLE 2.—*Influence of high initial percentages of total arsenious oxid upon oxidation.*

No. of test.	Per cent total As_2O_3 .	Per cent actual As_2O_3 by analysis.			
		Original.	10 days.	20 days.	30 days.
a.....	0.204	0.084	0.194	0.013
b.....	.244	.084	.226	.017	0.001
c.....	.284	.084	.261	.129	.002
d.....	.324	.084	.307	.179	.002
e.....	.364	.084	.314	.183	.002
f.....	.404	.084	.316	.189	.001

Experiment 3.—To determine the rapidity with which reduction may progress. Solution contained no actual As_2O_3 except that introduced in the used bath employed for inoculation, of which 10 per cent of final volume was added. Solution contained 0.242 per cent total arsenious oxid. A portion (test a) was placed in an entirely filled, tightly stoppered bottle, and another portion (test b) left open to the air as in experiments 1 and 2.

TABLE 3.—*Showing rapidity of reduction.*

No. of test.	Per cent actual As_2O_3 by analysis.			
	Original.	2 days.	11 days.	20 days.
a (sealed).....	0.021	0.183		
b (open).....	.021	.183	0.142	0.002

From a consideration of the results of experiments 1, 2, and 3, we may derive the following conclusions:

1. No amount of arsenic which can reasonably be used in a dipping bath is sufficient notably to retard the rate of oxidation or of reduction, though naturally a longer time is required for the complete oxidation of a large amount of arsenic than for a small amount.

2. Under conditions favorable to reduction the action may progress with extraordinary rapidity, far overbalancing any simultaneous opposing action of oxidizing organisms. But in such cases the reducing organisms comparatively soon exhaust either their vitality or the medium and thereafter are unable successfully to oppose the slow but persistent action of the oxidizing organisms.

One must proceed very cautiously in transferring any conclusions drawn from such laboratory experiments to the far different conditions which prevail in actual dipping baths in the field. None the less, taking all available data from field and laboratory into consideration, it is believed that a fairly accurate picture may now be drawn of the conditions which prevail in dipping baths in actual use. Suppose a vat to have been thoroughly cleaned and then charged with freshly prepared bath. All the arsenic will exist in the form of sodium arsenite and, if the bath be not used for dipping, will remain in that form except for a comparatively unimportant oxidation through purely chemical agencies. But as soon as the bath is used for dipping three additions are made to it: (1) oxidizing organisms; (2) reducing organisms; (3) nutrient matter upon which one or both of the varieties of microorganisms can subsist. At first, of course, the number of organisms are few and the nutrient is scanty. Under these conditions the reducing organisms do not thrive, but the oxidizing organisms are able to grow to a certain extent, and thus are able slowly to oxidize the bath. As further dipping is done in the vat, the bath becomes richer in nutrient matter, so that the oxidizing organisms flourish correspondingly and oxidize the arsenic with increasing rapidity. But after a time, if dipping is done in sufficient amount and at short intervals, the bath becomes so rich in nutrient matter that it finally forms a favorable medium for the growth of the reducing organisms, which then begin to flourish and appreciably to counteract the action of the oxidizing organisms. We thereafter

have two fully developed opposing forces in the vat—one tending to oxidize the arsenic, the other to reduce it. Which agency will gain the ascendancy at any given time will depend chiefly on the quantity and frequency with which cattle are being put through the bath. Frequent and abundant use of a bath will favor reduction; limited and infrequent use will favor oxidation. The oxidizing organisms must be conceived as working slowly but steadily and persistently under all conditions of use; the reducing organisms may work very rapidly when they work at all, but they work only spasmodically during ordinarily brief periods when the bath happens to be sufficiently rich in nutrient matter.

The next series of experiments were upon the effectiveness of certain antiseptics against the activity of both reducing and oxidizing organisms, with the double purpose of finding (1) a reliable means for preserving samples for analysis after removal from the vat and (2) a practical means of preserving the bath in the vat.

Experiment 4.—To determine if a layer of oil floated over the surface of the bath during periods of disuse would prevent oxidation. Mixture made in the usual way, placed in beakers, and covered with layers of oil of approximate thicknesses noted below.

TABLE 4.—*Effect on oxidation of oil floated over surface of bath.*

No. of test.	Per cent actual As_2O_3 by analysis.		
	Original.	6 days.	10 days.
a (no oil).....	0.255	0.185	0.003
b (1 mm. paraffin oil).....	.255	.219	.219
c (0.1 mm. paraffin oil).....	.255	.204	.009
d (no oil).....	.194013
e (1 mm. kerosene).....	.194033

Experiment 5.—On the efficacy of cresylic acid as an antiseptic against oxidation. Mixtures made in the usual way, certain proportions of cresol being added in the form of liquor cresolis compositus, U. S. P.

TABLE 5.—*Effect of cresylic acid as an antiseptic against oxidation.*

No. of test.	Per cent actual As_2O_3 by analysis.		
	Original.	6 days.	10 days.
a (no cresol).....	0.201	0.027
b (0.004 per cent cresol).....	.201	.106	0.006
c (0.02 per cent cresol).....	.201	.146	.003
d (0.10 per cent cresol).....	.201	.183	.180
e (0.50 per cent cresol).....	.201	.185	.181

Experiment 6.—On the efficacy of formaldehyde solution, U.S.P., as a preservative against oxidation. Carried out in open 300 c. c. Erlenmeyer flasks, earlier experiments having shown that working in open beakers led to misleading results owing to loss of formaldehyde by evaporation. Mixtures made and managed in usual way.

TABLE 6.—*Effect of formaldehyde solution as preservative against oxidation.*

No. of test.	Per cent actual As_2O_3 by analysis.		
	Original.	28 days.	71 days.
a (no formaldehyde solution).....	0.205	0.005
b (0.04 per cent formaldehyde solution).....	.205	.197	0.005
c (0.06 per cent formaldehyde solution).....	.205	.197	.191
d (0.08 per cent formaldehyde solution).....	.205193
e (0.10 per cent formaldehyde solution).....	.205192

Experiment 7.—On the efficacy of formaldehyde solution, U.S.P., as a preservative against reduction. Mixtures made in usual way and kept in entirely filled and tightly closed bottles. The content of the mixture in total arsenious oxid was approximately 0.21 per cent, and no actual arsenious oxid was added except that introduced through the bath used to inoculate the mixture.

TABLE 7.—*Effect of formaldehyde solution as preservative against reduction.*

No. of test.	Per cent actual As_2O_3 by analysis.			
	Original.	5 days.	12 days.	18 days.
a (no formaldehyde solution).....	0.015	0.208
b (0.02 per cent formaldehyde solution).....	.015	.013	0.013	0.210
c (0.03 per cent formaldehyde solution).....	.015013	.210
d (0.04 per cent formaldehyde solution).....	.015013

From these series of laboratory experiments it may be concluded:

(1) Flotation of a millimeter or so of paraffin oil upon the surface of dipping baths during periods of disuse may measurably retard oxidation. This it possibly does, not by forming a coating impermeable to the oxygen of the air, for oxygen is notably absorbed by petroleum oils,¹ but by retarding the rapidity of diffusion of oxygen into the bath underneath. Therefore oil might very possibly show to much better advantage in field experiments than in the above laboratory experiments, and doubtless deserves further study. Consideration must be given to the possibility of interference with thorough wetting of animals by a dip so treated and consequent failure to kill ticks, therefore the process should certainly not be used until

¹ Compare Comey's "Dictionary of Chemical Solubilities" and Seidell's "Solubilities of Inorganic and Organic Substances."

experiments which are not within the province of the writer have settled this point. It is of interest to note that a layer of oil will almost entirely prevent evaporation of water from the bath.

(2) Cresylic acid in admissible amounts only partially inhibits the growth of oxidizing organisms, a conclusion borne out by practical experience with proprietary dips containing that substance.

(3) Formaldehyde solution is a powerful and effective antiseptic against both classes of organisms, and inasmuch as it does not interfere with analytical methods for the determination of arsenic, it is appropriate as a preservative of samples of bath taken from a vat for chemical analysis, as recommended elsewhere by the writer.¹

Similar experiments were tried on some other antiseptics, especially those which would not interfere with analytical estimations of arsenic. Nothing was found, however, which at all approached formaldehyde in effectiveness and general applicability.

FIELD EXPERIMENTS OF 1914.

The experiments of 1914 were directed toward determining the efficacy and practical applicability of formaldehyde as a preservative against oxidation of dipping baths in the vat. Three vats were selected which were expected to be closely comparable in degree of usage, etc. One of the vats received no addition of formaldehyde and thus served as a check. The samples taken from the vats were further treated with formaldehyde and shipped in sealed bottles, while methods of analysis ¹ had by this time been thoroughly worked out. The analytical results of this series of experiments were very accurate, but are nevertheless best presented in the form of diagrams as in the case of the dipping experiments of 1913, no further explanation of the diagrams accordingly being necessary.

The diagram for vat I is very characteristic. Vat K, up to the twentieth week, shows what had been expected (from unpublished data) might occur if too little formaldehyde were used, namely, that the reducing organisms are more sensitive to it than are the oxidizing organisms, so that too little formaldehyde is worse than none at all. Vat J, as well as vat K after the twentieth week, shows that a sufficient amount of formaldehyde does decidedly decrease the extent to which oxidation progresses, especially after the vat has been in use for a few weeks, but for the first four to six weeks formaldehyde seems to be practically without effect in modifying the rate of oxidation. This is precisely the period during which the fresh antiseptic should be most effective against the thinly sown and scantily nourished organisms. It can only be concluded that in recently prepared baths the notable oxidation which may occur is in fact not chiefly the result of bacterial growth but of purely chemical processes. In

¹ Department of Agriculture Bulletin 76.

his first paper Brünnich¹ found that blowing air through solutions of sodium arsenite produced appreciable oxidation if pine tar or certain other substances were present, and Cooper and Freak² confirmed Brünnich's observations. From the present experiments the power of tar to promote oxidation seems to be exhausted in a few weeks, and thereafter the progress of oxidation, in accordance with the conclusions of Fuller, must be attributed to the growth of microorganisms.

GENERAL CONCLUSIONS.

The main conclusions already discussed in detail under the several series of experiments may be briefly summarized as follows:

(1) All used arsenical dipping baths may be expected to contain (a) oxidizing organisms which work slowly, but steadily and persistently, and (b) reducing organisms which work very rapidly at times, but spasmodically. The reducing organisms exert an appreciable effect only in vats which are used at frequent intervals for dipping large numbers of cattle. The ordinary vat, used once a fortnight, is likely to show only a slow, steadily progressing oxidation of the arsenic, and periodical analyses or tests³ must be made if proper dipping strength is to be maintained.

(2) Formaldehyde solution (37 per cent), used in the proportion of 1 gallon to every 1,500 gallons (8½ fluid ounces to 100 gallons) of liquid introduced into the vat, appears a safe and effective means for reducing oxidation to a low figure. But since there seems to be no evidence that under ordinary conditions oxidation is ever likely to progress so far as to result in the use of baths injurious to cattle, the question of the use of formaldehyde is purely economic. The writer believes that in most cases it will be cheaper to let some of the arsenic go to waste through oxidation. When the cost of a gallon of formaldehyde about equals the cost of all the materials necessary to make 500 gallons of dipping bath, there will probably be little financial gain either way, while there may be some real profit in its use through saving of labor in preparing dip and through the reduction of offensive odor from the bath by keeping it under antiseptic conditions.

¹ Australian Association for the Advancement of Science, 1909, vol. 12, p. 129.

² Journal of Agricultural Science, 1911, vol. 4, p. 177.

³ Department of Agriculture Bulletin 76.

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